AMENDMENTS TO THE DRAWINGS

Please cancel Figure 5, last panel, and Figure 7, panels A-C. In compliance with 37 CFR 1.121(d) and MPEP 608.02(t), applicants attach to this amendment the replacement sheet for Figure 5 labeled "Replacement Sheet" and a marked-up copy of the drawing sheet for Figure 7 including an annotation showing that the drawing has been cancelled and labeled "Annotated Sheet."

REMARKS

The present application is directed to methods of cloning genes using replication-deficient baculovirus vectors. Prior to this Amendment and Response, Claims 27-34 were pending. In the present Amendment and Response, applicants amend Claims 27, 29, and 30-34. The amendments do not introduce any new matter. Support for the amendment is found throughout the specification, for example, on p. 28, second paragraph, line 6, and in Claim 27 as originally filed. Applicants amend Claim 30 to bring it into correspondence with the previously submitted Claim 29. Support for the amendment is indicated in the Response to Final Office Action filed July 19, 2004. Upon entry of the present amendment, Claims 27-34 will be pending.

Drawings

The Examiner maintains the objection to Figure 5, last panel, and Figure 7, panels A-C, under 37 CFR 1.83(a), asserting that these drawings fail to show any details as described in the specification. Applicants request the Examiner to cancel Figure 5, last panel, and Figure 7, panels A-C. In compliance with 37 CFR 1.121(d) and 608.02(t), applicants attach to this amendment the replacement sheet for Figure 5 labeled "Replacement Sheet" and a marked-up copy of the drawing sheet for Figure 7 including an annotation showing that the drawing has been cancelled and labeled "Annotated Sheet." The changes to Figures 5 and 7 are presented in the section "Amendments to the Drawings."

In the section "Amendments to the Specification," applicants amend the brief description of the drawings in the specification so as to reflect the amendments to Figures 5 and 7. Applicants also renumber Figure 8 as Figure 7 and amend the paragraphs on pp. 25-26 of the specification in order to reflect the amendments to the drawings.

Applicants respectfully assert that the amendments to the drawings and to the specification overcome the objections to the drawings. Applicants request withdrawal of the objections to the drawings.

Claim Rejections under 35 U.S.C. § 112, second paragraph

The Examiner maintains the rejection of Claims 31-34 under 35 U.S.C. § 112, second paragraph, as indefinite. The Examiner states that, because of the double recitation of the term "functional gene" in Claim 29, it is not clear to which one of the "functional gene" terms the dependent Claims 31-34 are referring. Applicants amend Claim 29 to delete one of the terms "functional gene." Applicants also amend Claim 30, from which Claims 32 and 34 depend, to delete one of the terms "functional gene." Applicants assert that this amendment to Claims 29-30 overcomes the rejection of Claims 31-34 under 35 U.S.C. § 112, second paragraph, and request withdrawal of the rejection.

Claim Rejections under 35 U.S.C. § 112, first paragraph

The Examiner maintains the rejection of Claims 31-34 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. Applicants amend Claims 31-34.

The Examiner asserts that the applicants have not provided a disclosure sufficient to show that applicants were in possession of a genus of the functional fragments and mutations of the genes *lef 1-12, dnapol, p143, p35, ie-1-2, p47, ORF 1629* and *pp 31*. Applicants amend Claims 31-34 to delete the language "functional fragments and mutations." Applicants respectfully assert that the amendments overcome the rejection of Claims 31-34 under 35 U.S.C. §112, first paragraph, and request withdrawal of the rejection.

Claim Rejections under 35 U.S.C. §103(a)

Clark in view of Patel

The Examiner maintains the rejection of Claims 27-34 under 35 U.S.C. §103(a) as obvious over Clark *et al.* (hereinafter *Clark*), in view of Patel *et al.* (hereinafter *Patel*). Applicants re-assert their position that *Clark* or *Patel*, separately or in combination, fail to teach, suggest, or provide motivation to derive a method as recited in amended Claims 27-34. Applicants also re-assert their position that one of ordinary skill in the art would not be motivated to derive the claimed invention by combining the teachings of *Clark* and *Patel*.

Applicants disclose and claim a novel method of cloning genes using replication-deficient baculovirus vectors. This method possesses a number of unexpected advantages over known methods. Applicants discussed at least some of the advantages in the previous Responses.

Clark teaches a baculovirus **expression system with the apoptosis-resistance gene as the first selectable marker**. The parental vectors can only be maintained in an apoptosis-deficient cell line. *Clark* fails to teach or suggest use of a replication-deficient baculovirus vector that is capable of being maintained in non-insect (heterologous) cells. Thus, *Clark* fails to teach or suggest an element recited in amended Claims 27-34 and fails to render these claims obvious.

Patel teaches **recombination of baculovirus with a transfer vector in yeast** in order to simplify the selection of the recombinant virus. *Patel* fails to teach **replication-deficient** baculovirus vectors, and fails to teach, suggest, or provide motivation for transfecting insect cells with a replication-deficient baculovirus vectors. Insect cells in *Patel* are transfected by recombinant, **replication-capable** baculovirus, which was obtained from the yeast cells. See, for example, *Patel*, page

103, beginning of column 1. *Patel* fails to teach or suggest an element recited in amended Claims 27-34 and fails to render these claims obvious.

Applicants re-assert that one of ordinary skill in the art **would not** be motivated to combine the teachings of *Clark* and *Patel* to derive the applicants' invention, as claimed. Furthermore, modification of the teachings of *Clark*, as asserted by the Examiner in this and the previous Office Actions, would not result in a method possessing the advantages of applicants' invention, and it therefore fails to render applicants' method obvious.

Kitts in view of Patel

The Examiner maintains the rejection of Claims 27-34 under 35 U.S.C. §103(a) as obvious over Kitts *et al.* (hereinafter *Kitts*) in view of *Patel.* Applicants re-assert their position that *Kitts,* separately or in combination with *Patel*, fails to teach, suggest or provide motivation to derive applicants' invention, as currently claimed, and fails to render it obvious. Applicants reassert their position that one of ordinary skill in the art would not be motivated to derive the claimed invention by combining the teachings of *Kitts* and *Patel.*

Kitts teaches a method of producing a recombinant baculovirus vector in insect cells that uses **baculovirus DNA linearized with a restriction enzyme** in combination with standard transfer vectors. See, for example, Figure 1 in *Kitts*. *Kitts* fails to teach, suggest, or provide motivation to use of an intermediate host. *Kitts* fails to teach, suggest, or provide motivation to derive a baculovirus vector that is replication-deficient prior to linearization. *Kitts* fails to teach or suggest an element recited in amended Claims 27-34 and fails to render these claims obvious.

The teachings of *Patel* are discussed in the previous section. Applicants re-assert that one of ordinary skill in the art **would not** be motivated to combine the teachings of *Kitts* and *Patel* to derive the applicants' invention, as claimed.

Furthermore, modification of the teachings of *Kitts*, as suggested by the Examiner in this and previous Office Actions would not result in a method possessing the advantages of applicants' invention, and therefore fails to render applicants' method obvious.

In view of the foregoing, applicants respectfully request the Examiner to withdraw the rejections of Claims 27-34 under 35 U.S.C. §103(a) over *Clark* in view of *Patel* and over *Kitts* in view of *Patel.*

Claim amendments

Applicants respectfully assert that the claim amendments overcome the rejections of claims under 35 U.S.C. §103(a) as obvious over *Clark* in view of *Patel* and over *Kitts* in view of *Patel.* Applicants further amend Claim 27 to recite **circular, replication-deficient** baculovirus vectors. Applicants also amend Claim 27 to recite circular replication-deficient baculovirus vectors capable of being maintained **in a bacterial host**. Support for the amendment is found throughout the specification, for example, on p. 28, second paragraph, line 6, and in Claim 27 as originally filed. Applicants assert that amendment to Claim 27 overcomes the rejections.

Clark teaches **linearized, replication deficien**t baculovirus vectors. In *Clark*, the vectors are linearized by restriction enzymes. See, for example, *Clark*, Figure 2 (bottom left drawing) and column 13, lines 29-31. **Linearization of the vector within the ORF1626 sequence** renders the vector replication-deficient. The viability of the viral DNA is restored by recombination with the rescue vector in the insect cells. *Clark* fails to teach, suggest, or provide motivation to derive a vector capable of replicating in a bacterial host. *Clark* fails to teach or suggest a method recited in amended Claims 27-34 and fails to render these claims obvious.

Kitts teaches using **baculovirus DNA linearized with a restriction enzyme** in combination with standard transfer vectors. *Kitts* fails to teach , suggest, or provide motivation for transfecting insect cells with a circular, replication-deficient baculovirus vector. *Kitts* fails to teach, suggest, or provide motivation to derive a vector capable of replicating in a bacterial host. *Kitts* fails to teach or suggest a method recited in amended Claims 27-34 and fails to render these claims obvious.

Patel fails to teach or suggest circular, replication deficient baculovirus vectors. *Patel* teaches vector recombination and selection in yeast. Therefore, there is no need in the *Patel* system to render the vectors replication deficient in insect cells. *Patel* teaches recombination of baculovirus with a transfer vector **in yeast** in order to simplify the selection of the recombinant virus. *Patel* fails to teach replication-deficient baculovirus vectors capable of replicating **in a bacterial host**. Thus, *Patel* fails to teach or suggest an element recited in amended Claims 27-34 and fails to render these claims obvious.

Moreover, *Patel* teaches **replication and recombination** of the baculovirus vectors **in the yeast cells,** but fails to teach replication of a defective baculovirus genome in a bacterial cell, followed by recombination in the yeast cells, as recited in Claims 27-34. Prior to applicants' present invention, it was not known or obvious to one of ordinary skill in the art that replication-deficient baculovirus vectors could be maintained in bacteria, and then undergo subsequent recombination in the insect cells with the homologous baculovirus sequences. Support for this assertion is provided in the Second Declaration under 37 C.F.R. §1.132 (hereinafter referred to as "the Second Declaration") by Dr. Robert David Possee, an expert in the field of the invention and a named inventor of the present patent application (see Section 2 of the Second Declaration).

In view of the foregoing, applicants respectfully request the Examiner to withdraw the rejections of Claims 27-34 under 35 U.S.C. §103(a) over *Clark* in view of *Patel* and over *Kitts* in view of *Patel.*

Examiner's Reply to Applicants' Arguments and Declaration Previously Submitted in Response to the Rejections of Claims under 35 U.S.C. §103(a)

On page 11 of the Final Office Action, the Examiner replied to the arguments and Declaration under 37 C.F.R. §1.132 (hereinafter referred to as "the First Declaration") by Dr. Robert David Possee that were previously submitted to address the rejections of Claims under 35 U.S.C. §103(a). Below, applicants respond to the Examiner's reply. Applicants use the order and the numbering system used by the Examiner in the Office Action. Additional support for applicants' arguments is provided in the Second Declaration.

1. In the First Declaration, applicants argued that their method, as compared to *Clark,* results in lower contamination with the parental baculovirus. The Examiner states that applicants did not support the claims by factual evidence, only objective arguments. The Examiner asserts that it is the burden of the applicants to demonstrate that the difference in the result is significant.

Applicants provide the supporting factual evidence in the Second Declaration. See Section 3 of the Second Declaration and Exhibit A. Exhibit A includes Figures 1 and 2 and their legends. Figures 1 and 2 show that when applicants' novel method is used to make recombinant baculoviruses, the recombinant viruses are produced with observable 100% efficiency and without any observable contamination by the parental virus. In particular, Figures 1 and 2 show that co-transfection of all forty four wells of Sf9 cells with applicants' baculovirus vectors and transfer vectors containing a gene to be cloned resulted in production of a recombinant protein.

Figure 5 in *Clark* shows an example of producing recombinant baculovirus with a ninety six-well plate system. Sf9 cells were co-transfected with the parent virus DNA and DNA containing a foreign gene. *Clark* states that the resulting recombinant virus was passaged three times on Sf9 cells to amplify and normalize viral titers. When cells from the final passage were stained with Magenta-gluc to detect the presence of recombinant baculovirus, only 78 out of 88 experimental wells (or 89% of the wells) contained the recombinant virus. See brief description of Figure 5, starting in column 6, line 59. This shows that **the *Clark* system was observed to be less than 100% efficient,** and that, most likely, the parental virus contaminated the recombinant virus stocks.

In contrast, when applicants' method is used to make recombinant viruses, **the recombinant viruses are produced with observable 100% efficiency** and without any observable contamination by the parental virus.

2(a). The Examiner states that applicants provided no evidence that the vector in *Clark* is not capable of being maintained in an intermediate host, such as yeast or bacteria. The Examiner also states that a minimal requirement for maintaining a vector in a yeast or a bacterial host is for the yeast or bacteria to be able to be transformed with the vector.

Applicants provide the supporting factual evidence in the Second Declaration. See Section 4 of the Second Declaration. Applicants disagree that a minimal requirement for maintaining a vector in a yeast or a bacterial host is for the vector DNA to be **transformed** by yeast or bacteria. Applicants respectfully bring to the Examiner's attention that a vector must comprise a yeast or bacterial origin of replication and an appropriate selectable marker to be **maintained** in an intermediate host. *Clark* fails to teach or suggest elements necessary for replication and/or transformation in yeast or bacteria.

Applicants conducted experiments in which vectors substantially similar to the *Clark* vectors were introduced into yeast or bacterial cells by LiCl-mediated transformation or electroporation, respectively. Yeast or bacterial cells failed to support replication of the baculovirus DNA when a yeast-specific or a bacteria-specific replicon was not present in the virus genome. Thus, a vector in *Clark* is not capable of being maintained in an intermediate host, such as yeast or bacteria at least because *Clark* fails to teach, suggest, or provide motivation to include in its vectors a yeast-specific or a bacteria-specific replicon.

2(b). The Examiner states that, in the previous Response, applicants argued that the viral vector in *Clark* is only suitable for use in the apoptosis deficient host cell line (*T. ni*), but that applicants have not supported these arguments by actual proof.

Applicants assert that previously known experimental data supports their conclusions. Applicants provide additional supporting evidence in the Second Declaration. See Section 5 of the Second Declaration.

In the previous Response, applicants asserted that: (a) the use of such cell line creates a heterogeneous population and reduces production of the desired protein; (b) the vector in *Clark* cannot be grown in any intermediate host other than the apoptosis deficient insect cell line. If the *Clark* or similar vectors are introduced into the cells other than an apoptosis-deficient cell line, such as *T. ni*, then virus yields are reduced from about 10^7 plaque forming units (pfu) per ml to about 10^4 pfu/ml, as shown independently by Lerch and Friesen (1993). See Table 1 in Lerch and Friesen (1993), Exhibit B.

This has the following consequences for viral DNA production. If the *Clark* vectors are amplified in *T. ni* cells, then virus production will be nearly the same as for unmodified baculovirus in Sf9 cells. However, if the *Clark* vectors are amplified in Sf9 cells, which are susceptible to apoptosis, virus yields will be reduced 1000-fold. When purifying viral DNA, it is extracted from the virus particles released from

insect cells. Normally, about 100 μg of viral DNA from 500 ml of virus-infected Sf9 cells is expected. With a 1000-fold reduction in the starting material, the *Clark* system, will yield approximately 0.1 μg of viral DNA from 500 ml of cell culture. This amount of viral DNA is sufficient for transfection of only two cultures of insect cells in order to make recombinant viruses. **Twenty four liters** (24 L) of virus-infected cell culture will be needed in order to produce 96 viruses in the high-throughput 96-well plate system, as shown in Figure 5 in *Clark*. Due to the excessive time and labor that will be involved, such large amounts of insect cells culture will be impractical to use for purification of the virus DNA. Thus, only apoptosis deficient cells, such as *T. ni* cells, are, in fact, suitable for parental virus production of *Clark* vectors. The disadvantages of using *T. ni* cells were discussed in the previous Responses and include tendency to mutations that reduce very late gene expression.

2(c). The vector in *Clark* is a double mutant with the deletion in the ORF1629 gene, which is one of the genes recited in Claims 31 and 32. Applicants asserted in previous Responses, that the *Clark* system, unlike the system in the claimed method, is subject to contamination by the parental vector. The present application states that the mutation in the ORF 1629 gene is adequate to prevent parental contamination of the virus. The Examiner asserts, that, based on the disclosure of the present application, the ORF 1629 mutation in *Clark* should also prevent the contamination by the parental vector. Applicants disagree. Applicants provide additional support for their arguments below in the Second Declaration. See Section 6 of the Second Declaration.

Applicants respectfully bring to the Examiner's attention that *Clark* does not teach, suggest, or provide motivation to use a mutation in ORF1629 to render its vector replication-deficient. Modifications in ORF1629 in *Clark* are additional restriction sites, but they do not render the vector replication-deficient (see column 13, lines 24-26). *Clark* employs the technique of Kitts and Possee (1993), which uses linearization by a restriction enzyme of a parental virus. In *Clark*, a full-length copy

of ORF1629 is present in the replication-capable parental virus genome prior to linearization, and **the deletion in ORF1629 is achieved by enzymatic digestion with a restriction enzyme**. The restriction within ORF1629 renders the virus both linearized and replication-deficient. See, for example, Figure 2 in *Clark*. **But this deletion is not capable of preventing the parental contamination**.

As applicants previously discussed, this technique possesses a number of disadvantages over applicants' system. One of these disadvantages is contamination by the parental virus as a result of incomplete digestion by a restriction enzyme. Due to incomplete digestion, the linearized replication-deficient virus will be contaminated with the parental replication-capable virus comprising a functioning ORF1629. This undigested circular DNA will result in contamination by the parental virus.

In applicants' invention, on the other hand, the parental virus is replication-deficient because it has a deletion in ORF1629 in a circular viral genome. The replication-deficient viral DNA is used to co-transfect Sf9 cells **without pre-digestion with a restriction enzyme**. Thus, in applicants' system the source of contamination by an undigested parental virus is eliminated.

2d. The Examiner states that *Clar*k does not limit the replication of its virus to the insect cells. Applicants disagree. Applicants provide additional support for their arguments below in the Second Declaration. See Section 7 of the Second Declaration.

Applicants respectfully bring to the Examiner's attention that applicants' invention comprises **replication in bacterial cells of baculoviruses that are replication-deficient in insect cells**, not replication in bacterial cells in general. However, *Clark* fails to teach, suggest, or provide motivation for virus maintenance in cells other than insect cells. Since *Clark's* baculoviruses are not replication-deficient in insect cells until they are linearized, one of ordinary skill in the art would not be motivated to replicate them in cells other than insect cells. Accordingly, *Clark* fails to

teach, suggest or provide motivation for introducing into the baculovirus vectors elements necessary for replication in the heterologous cells. Thus, *Clark* effectively limits the replication of its virus to the insect cells.

The Examiner states that *Clark* suggests using heterologous hosts, for example, in column 3, line 1-3, where *Clark* summarizes the strategies involving baculovirus recombination in a heterologous host. However, in the preceding paragraphs *Clark* characterizes the strategies involving heterologous hosts as cumbersome and complicated (see column 2, lines 43-44 and 66-67). Therefore, *Clark*, in fact, **teaches away** from using heterologous hosts. In any case, the strategies discussed in *Clark* in column 2, lines 26-67, involve recombination of baculovirus in heterologous hosts, not replication of a circular replication-deficient baculovirus in a heterologous host, as in currently claimed applicants' method.

The Examiner also refers to column 6, line 9, where *Clark* discusses suitable hosts. The Examiner states that *Clark* does not limit the hosts to insect cells. Applicants respectfully bring to the Examiner's attention that *Clark* uses insect cells as the only example of a suitable host in column 6, line 9. The paragraph in column 11, line 50-66, to which the Examiner refers, deals with the general definition of the term "host" and does not specifically refer to the *Clark* system.

There is no motivation provided in *Clark* to introduce a heterologous host into the *Clark* system, and one of ordinary skill in the art would not be motivated to use a heterologous host because there is no advantage in doing so in the *Clark* system. See Section 8 of the Second Declaration for additional support. *Clark* constructs comprise a p35 mutation and additional restriction sites in ORF1629, which **do not prevent replication of the parental virus in the insect cells unless the vectors are digested with a restriction enzyme prior to transfection**. If, given a benefit of a hindsight, *Clark* was motivated to use a heterologous host, there would have been be no need to include an additional step of digesting the vectors prior to transfection into

the insect cells (which are, in fact, the only cells suggested by *Clark*). Furthermore, applicants' amended claims recite "circular vectors," in contrast to *Clark*, which uses linearized vectors for transfection.

3. The Examiner states on p. 14, that applicants' previously filed arguments with respect to *Kitts* are not persuasive. *Kitts* teaches linearization of baculovirus vectors by restriction enzymes in order to render them replication-deficient. The method in *Kitts* results in lower yields of the recombinant virus than applicants' method, because a small proportion of the undigested viruses always remains intact. The Examiner asserts that the claims are neither limited to the circular vectors, nor to the yield of the recombinant vectors. Amended claims recite "circular vectors," thereby rendering moot the Examiner's assertion.

4. The Examiner asserts, on p. 15 of the Office Action, that the combinations of *Patel* and *Clark,* and *Patel* and *Kitts* render applicants' invention obvious, and that *Patel* contains the motivation to combine the elements of *Clark* or *Kitts* with *Patel*. Applicants respectfully assert that *Clark* or *Kitts*, separately or in combination with *Patel,* respectively, fail to teach, suggest, or provide motivation to derive an element of applicants' invention as recited in the amended claims – **a circular, replication deficient baculovirus vector**.

Applicants discuss *Clark*, *Kitts,* and *Patel* in the appropriate sections of the Response. *Patel* teaches recombination of baculovirus with a transfer vector **in yeast** in order to simplify the selection of the recombinant virus. But *Patel* fails to teach a vector capable of replicating in a **bacterial host**. In addition, applicants re-assert that ***Patel* fails to teach replication-deficient baculovirus vectors**, and fails to teach, suggest, or provide motivation for transfecting insect cells with a replication-deficient baculovirus vectors. Insect cells in *Patel* are transfected by recombinant, **replication-capable** baculovirus, which was obtained from the yeast cells. See, for example,

Patel, page 103, beginning of column 1. *Patel* fails to teach or suggest an element recited in amended Claims 27-34 and fails to render these claims obvious.

In view of the claim amendments and the foregoing arguments, applicants respectfully request withdrawal of the rejections of Claims 27-34 under 35 U.S.C. §103(a) over *Clark* in view of *Patel* and over *Kitts* in view of *Patel.*

CONCLUSION

Applicants respectfully submit that this is a complete response to the Final Office Action dated April 19, 2005. Applicants respectfully assert that the claims are now in condition for allowance and request that the application be passed to issuance. If the Examiner believes that any informalities that may be corrected by Examiner's amendment remain in the case, or if there are any other issues which can be resolved by a telephone interview, a telephone call to the undersigned agent at (404) 815-6102 is respectfully solicited.

Respectfully submitted,

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ANNOTATED SHEET

THIS DRAWING HAS BEEN CANCELLED.

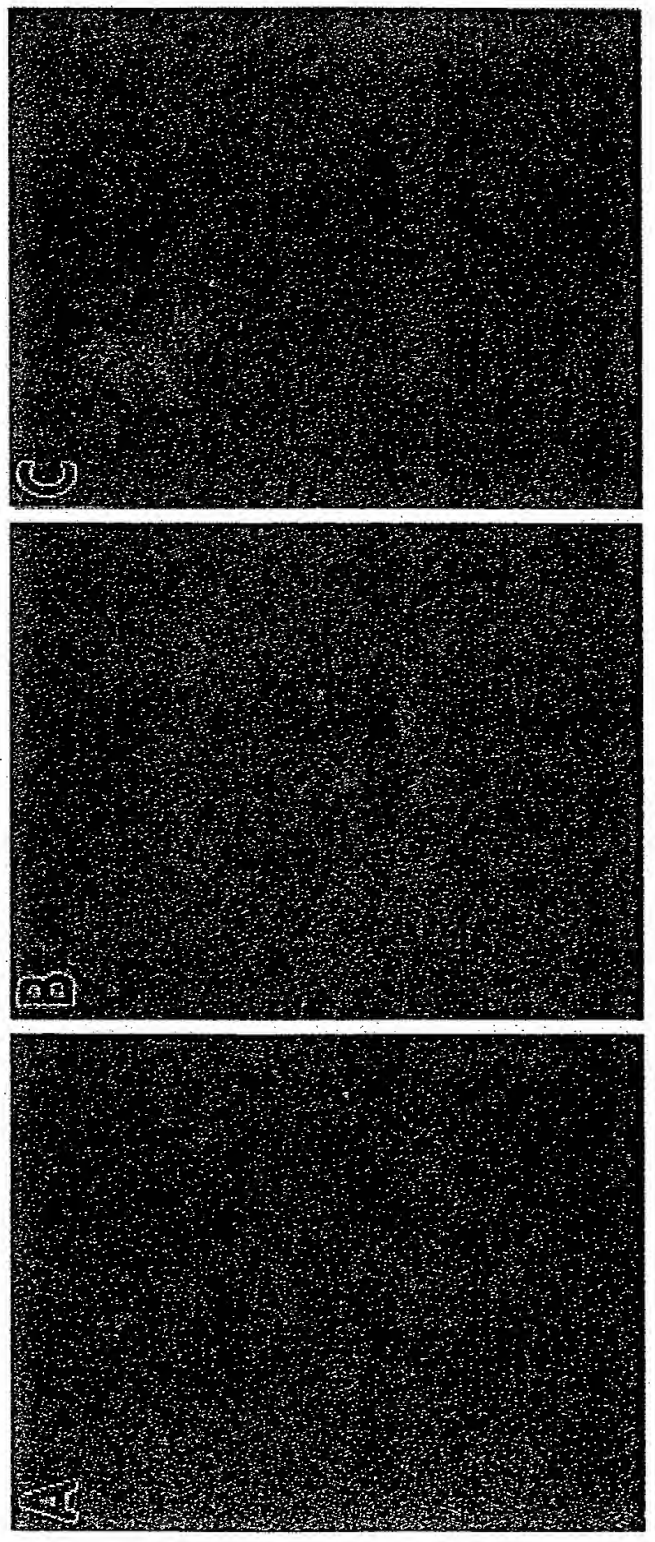

FIG. 7

REPLACEMENT SHEET

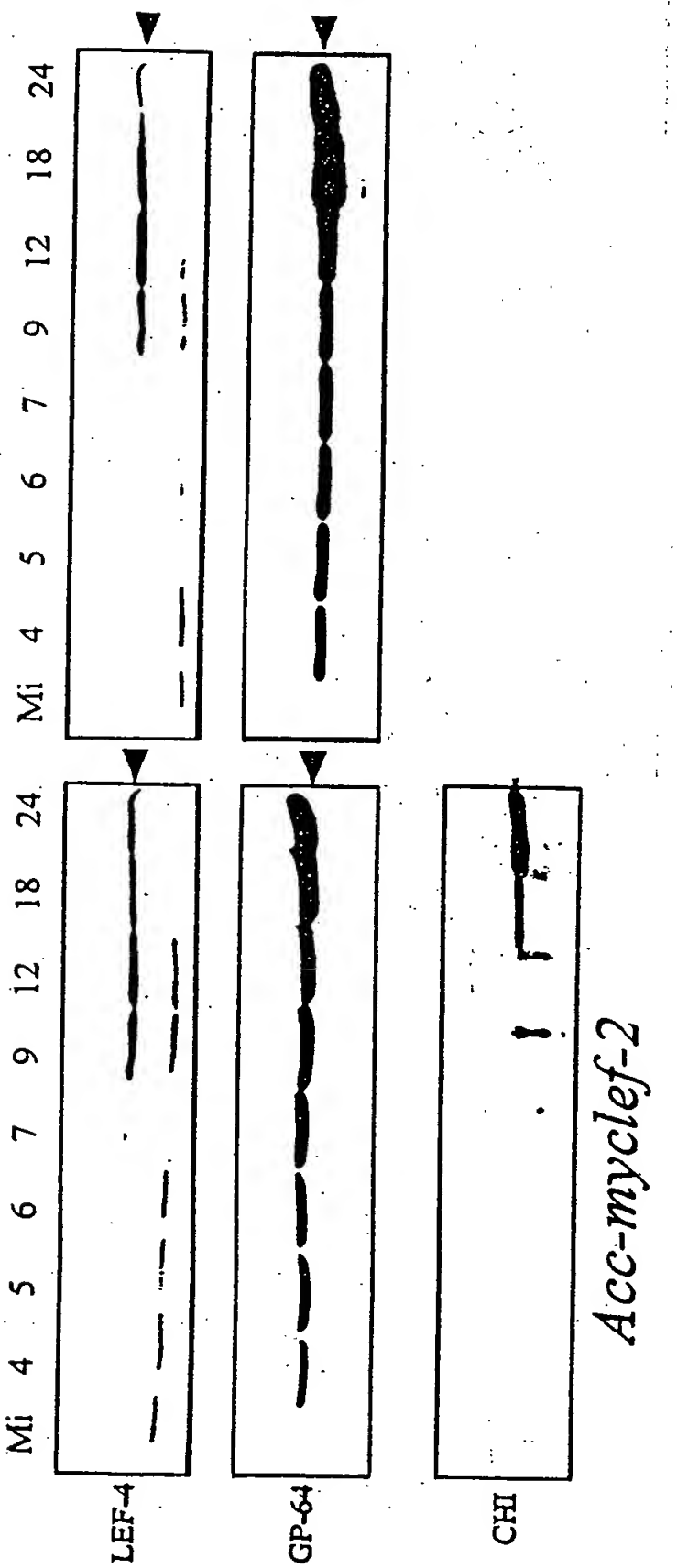

Figure 5